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Is there rapid evolutionary response in introduced populations of tansy ragwort, *Jacobaea vulgaris*, when exposed to biological control?

Carole Rapo · Heinz Müller-Schärer · Klaas Vrieling · Urs Schaffner

Abstract Differences in the herbivore community between a plant's native (specialists and generalists) and introduced range (almost exclusively generalists) may lead to the evolution of reduced allocation to defences against specialist herbivores in the introduced range, allowing for increased allocation to competitive ability and to defences against generalist herbivores. Following this logic, the introduction of biological control agents should reverse this evolutionary shift and select for plants with life-history traits that are more similar to those of plants in the native range than those of plants in the introduced range that have not been exposed to biological control. In a common garden experiment, we compared performance and resistance traits of tansy ragwort, *Jacobaea vulgaris,* among populations from the introduced range (New Zealand and North America) that have either been exposed to or grown free from the biological control agent *Longitarsus jacobaeae*. For comparison, we included populations from the native European range. We found lower levels of generalist-deterrent pyrrolizidine alkaloids (PAs) and of soluble phenolics in New Zealand populations with than in populations without exposure to *L. jacobaeae*, while the opposite pattern was detected among North American populations. Contrary to expectation, populations with exposure to *L. jacobaeae* revealed more feeding damage by *L. jacobaeae* than populations without exposure. Introduced populations had higher levels of PAs and reproductive output than native *J. vulgaris* populations. *Jacobaea vulgaris* was introduced in different parts of the world some 100–130 years ago, while *L. jacobaeae* was introduced only some 20–40 years ago. Hence, the larger differences observed between native and introduced populations, as compared to introduced

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populations with and without biological control history, may result from different time scales available for selection to act.

Keywords Biological control · Evolution of increased competitive ability (EICA) · Invasive alien species · Reproductive output · Pyrrolizidine alkaloids · Phenolics

Introduction

Various hypotheses have been put forward to explain the increased invasive potential of exotic plant species (Elton 1958; Alpert et al. 2000; Colautti et al. 2004; Mitchell et al. 2006). Several of these hypotheses are based on the assumption that plants are exposed to different sets of natural enemies in the native and the introduced range. This assumption has been supported by numerous studies showing higher richness and different composition of natural enemies associated with invasive plant species in their native range as compared to their introduced range (cf. reviews by Keane and Crawley 2002; Mitchell and Power 2003; Torchin et al. 2003; Mitchell et al. 2006). The Enemy Release hypothesis (ERH; Keane and Crawley 2002) proposes that plant species, on introduction into a new range, experience a reduction in top–down regulation by natural enemies that enables them to increase in abundance and out-compete the native plants in the introduced range (Maron and Vilà 2001; Mitchell and Power 2003; Torchin et al. 2003). The Evolution of Increased Competitive Ability hypothesis (EICA; Blossey and Nötzold 1995), an evolutionary extension of the ERH, is based on the assumption of a trade-off between growth and defence (Herms and Mattson 1992; Stamp 2003) and provides an additional mechanism for plant invasion. Under conditions of reduced herbivory in the introduced range, the EICA hypothesis states that selection will favour genotypes with higher allocation towards competitive abilities and growth, and lower allocation towards defence traits, as defence no longer increases fitness.

When testing the EICA hypothesis, it is necessary to incorporate the fundamental difference between specialist (feeding on one or a few closely related plant species) and generalist (feeding on several non-related plant species) herbivores (Müller-Schärer et al. 2004; Joshi and Vrieling 2005). The specialist-generalist dilemma (van der Meijden 1996) argues that in the native range, intermediate levels of plant toxins are maintained by opposing selective forces of adapted specialists that use plant defence chemicals as host-plant recognition cues and feeding or oviposition stimulants, and of non-adapted generalists that are deterred by the same chemicals. The allocation costs of plant toxins such as alkaloids or glucosinolates appear to vary considerable, but often tend to be moderate (Strauss et al. 2002). In contrast, digestibility-reducing metabolites such as lignin act as defences against both generalist and specialist herbivores, and they occur at relatively high concentrations and incur significant allocation costs (Müller-Schärer et al. 2004). By combining the EICA hypothesis and the specialist-generalist dilemma, one may hypothesize that plants introduced into areas where specialist herbivores are lacking but generalist herbivores are present may evolve increased levels of defence traits that act primarily against generalist herbivores, and decreased levels of defence traits that act also against specialist herbivores. If the defence chemicals against generalists are less expensive than those against the specialists, then an evolutionary shift in defence strategy in invasive populations may result in a net gain of resources for the plant, which then can be invested into increased growth and reproduction (Müller-Schärer et al. 2004; Joshi and Vrieling 2005).

So far, studies comparing life-history traits of populations from the native and the invaded range have revealed mixed results (for references see Müller-Schärer et al. 2004; Bossdorf et al. 2005; Maron and Vilà 2008). One reason for this may be that testing the EICA hypothesis is not a simple task. A thorough test requires knowledge of the origin of the invasive populations to make sure that differences in means of life-history traits do not simply reflect the introduction of a subset of genotypes from the native range with some specific characters. Moreover, differences in growth rate, reproductive output or defence traits that may be observed in cross-continent comparisons may also have evolved in response to factors other than those related to herbivory (Colautti et al. 2004, 2009). For example, Maron et al. (2004, 2007) found latitudinally based clines in leaf traits, growth and reproductive output in *Hypericum perforatum* L. in the introduced range in North America.

An alternative way to improve our understanding of evolutionary changes in introduced populations in response to different herbivore assemblages is to compare life-history traits of populations within the introduced range that have experienced successful biological control with those of populations that have not been exposed to classical biological control (Maron and Vilà 2008; Müller-Schärer and Schaffner 2008). Following the same lines of argumentation as the EICA hypothesis, and considering the specialist-generalist dilemma, populations that have been reunited with specialist herbivores through biological control measures should evolve genotypes with increased defences against specialist herbivores, reduced defences against generalist herbivores and decreased growth rate and/or reproductive output. Hence, populations exposed to biological control should become more similar to native populations than to populations from the introduced range that have not been exposed to classical biological control.

From the studies published so far (reviewed in Hinz and Schwarzlaender 2004; Bossdorf et al. 2005), some of the best evidence for both higher susceptibility to herbivory and higher competitive ability in the introduced range come from species that were introduced to the new area 200–250 years ago, and that therefore had the opportunity to adapt to the new conditions over a relatively long time period (Dietz and Edwards 2006). In contrast, most of the classical biological control programs against invasive plant species are only 20–50 years old (Julien and Griffiths 1998). Despite the relatively short exposure time of most invasive plant species to biological control organisms, we believe that comparison of invasive populations with and without a biological control history offers new opportunities to test the evolutionary trajectories of plant species in the presence and absence of specialist natural enemies (Joshi and Vrieling 2005; Handley et al. 2008). It is expected that invasive plants experience especially strong selection from specialist herbivores in successful biological control projects (Crawley 1983; McEvoy et al. 1991; Crutwell McFadyen 1998; Maron and Vilà 2008; Zangerl et al. 2008).

Tansy ragwort, *Jacobaea vulgaris* Gaertn., is a toxic biennial or short-lived perennial native to Eurasia and introduced to different parts of the world in the late nineteenth and early twentieth centuries (Coombs et al. 1999) where it became a serious weed problem affecting livestock production, crop and forage yields. In the 1960s, a biological control programme was launched against *J. vulgaris* that resulted in the release of several biological control agents in North America, New Zealand and Australia. In some regions, biological control agents brought *J. vulgaris* under control, while in other regions the weed continues to spread (Julien and Griffiths 1998; Coombs et al. 2004).

Previous studies revealed that populations of *J. vulgaris* from the introduced range have higher levels of pyrrolizidine alkaloids (PAs), a higher growth rate, a higher reproductive output and a decreased resistance against specialist herbivores, such as *Longitarsus*

jacobaeae (Waterhouse; Coleoptera, Chrysomelidae) and *Tyria jacobaea* L. (Lepidoptera, Arctiidae), as well as increased resistance against generalist herbivores (Joshi and Vrieling 2005; Stastny et al. 2005). Here we set out to assess whether introduced *J. vulgaris* populations that have been re-exposed to the biological control agent *L. jacobaeae* differ from those populations from the introduced range that have never been exposed to classical biological control of *L. jacobaeae*. In a common garden experiment in the native range we grew populations of *J. vulgaris* originating from two different parts of the introduced range, i.e. North America and New Zealand, and compared relative growth rate (RGR) and reproductive output. To assess defence traits, we measured leaf dry matter content (LDMC) and concentration of soluble phenolics (as indicators for resistance against specialist and generalist herbivores), concentration of PAs (as indicator for resistance against generalist herbivores) as well as resistance to the specialist *L. jacobaeae,* which occurs naturally at the study site. For comparison, we also included *J. vulgaris* populations from the native European range. We predicted that populations in the introduced range that have experienced biological control by *L. jacobaeae* will (1) grow smaller, (2) show lower reproductive output, (3) harbour lower levels of resistance against generalist herbivores, and (4) have higher levels of resistance against specialist herbivores than introduced populations that have not been exposed to biological control. With regard to these plant traits, we furthermore predicted that (5) populations from the invaded range that have been exposed to *L. jacobaeae* are more similar in terms of growth, reproduction and defence traits to the native European populations than to introduced populations without biological control management by *L. jacobaeae*.

Materials and methods

Jacobaea vulgaris and its biological control history

Jacobaea vulgaris has been previously named *Senecio jacobaea*, but new taxonomical insights place it in a separate genus *Jacobaea* (Pelser et al. 2002, 2006). This plant and its associated herbivores are among the most extensively studied systems in plant-insect interactions (e.g. Dempster and Lakhani 1979; van der Meijden and van der Waals-Kooi 1979; Myers 1980; McEvoy et al. 1991). In its native range, which extends from western Europe to Central Asia, *J. vulgaris* is attacked by more than 70 herbivores, several of which have a very narrow host-range (Harper and Wood 1957). In North America, some 40 native, predominately generalist arthropods have been recorded feeding on *J. vulgaris*, with unknown impact on the plant's population dynamics (Frick 1972). *Jacobaea vulgaris* has also been intensively studied in terms of its chemical defence systems, especially the production of PAs (Hartmann and Witte 1995), which are secondary compounds deterrent to non-adapted (generalist) herbivores (van Dam et al. 1995; de Boer 1999) but attractive to the specialist herbivore *Tyria jacobaea* (Macel and Vrieling 2003). Previous studies provide evidence that variation in concentration and composition of PAs are genetically based (Vrieling et al. 1993).

Besides PAs, *J. vulgaris* also contains a number of soluble phenolics such as chlorogenic acid and flavonoids (Kirk et al. 2005) Generally, soluble phenolics are assumed to act as defences against both generalist and specialist herbivores (Schoonhoven et al. 1998; Stamp and Osier 1998; Müller-Schärer et al. 2004), and this has also been shown for chlorogenic acid (Bernays et al. 2000; Leiss et al. 2009). We therefore consider the soluble phenolics in *J. vulgaris* to act as potential defences against generalist and specialist

herbivores. Moreover, leaf dry matter content (leaf dry weight divided by leaf fresh weight) was used as a proxy for quantitative chemical defences that reduce digestibility for both specialist and generalist herbivores (Elger and Willby 2003), because this trait is closely related to total phenolics (which includes lignin and tannins; Hanley and Lamont 2002).

Jacobaea vulgaris was first recorded in New Zealand in 1874 (Thomson 1922), in Canada in 1913 (Harris et al. 1971) and in the USA in 1922 (Isaacson 1973). Six biological control agents have been introduced against *J. vulgaris* in North America, Australia or New Zealand (Julien and Griffiths 1998). Studies by McEvoy et al. (1991) and McEvoy (1999) provide evidence that the successful control of tansy ragwort in western US is largely due to the introduction of the flea beetle *Longitarsus jacobaeae*. Adult beetles feed on the foliage throughout the summer and fall, leaving characteristic feeding punctures (hereafter called "shot-holes"), and the larvae feed inside the roots. This beetle sequesters PAs (Dobler et al. 2000) and may therefore benefit from feeding on PA-rich plants, but it is unknown whether it uses PAs in locating its host-plant. *Longitarsus jacobaeae* was introduced from Italy to northern California in 1969 (Frick 1970) and to Oregon in 1971 (Isaacson 1978). A consignment of *L. jacobaeae* beetles was subsequently sent from Oregon to New Zealand where first field releases were made in 1983 (Syrett et al. 1984). *Jacobaea vulgaris* was brought under successful biological control in northern California by 1976 (Pemberton and Turner 1990) and in western Oregon in the early 1980s (McEvoy et al. 1991; Coombs et al. 2004). Between 1983 and 1999, 158 populations of *L. jacobaeae* beetles were released in different regions of New Zealand. Successful and ongoing control of *J. vulgaris* has been reported from a number of sites where *L. jacobaeae* got established. However, the weed continues to thrive in areas where the beetle was not released or failed to establish (Hayes 2000). Besides *L. jacobaeae*, the cinnabar moth, *Tyria jacobaeae*, which attacks the above-ground plant parts, is considered to have at least regionally contributed to the biological control of *J. vulgaris* populations as well (Julien and Griffiths 1998).

Sampled plant material

Seeds of 32 populations from the native and the introduced range of *J. vulgaris* were collected between 2002 and 2004 and stored at 5°C. From each population, between 10 and 20 seed families (maternal plants) were sampled and bagged separately. Local experts provided information on whether *L. jacobaeae* had successfully established and become abundant at least during a certain period in time, or whether it had not been released or failed to establish at the sampling site. In the first case, the *J. vulgaris* population was classified as having had exposure to *L. jacobaeae*, while in the two latter cases the population was classified as having had no exposure to *L. jacobaeae*. Care was taken to omit populations from which only low densities of *L. jacobaeae* had been reported, because populations that had never been exposed to high densities of the biological control agents are unlikely to have experienced significant selection by this species. All populations from the native range harboured populations of *L. jacobaeae* or the sibling species *Longitarsus flavicornis* L. No phylogenetic study on the origin of invasive *J. vulgaris* has been published so far. It is therefore unknown whether the selected populations from the native range (Switzerland and The Netherlands) are representative of all native populations, and whether they are from the range from which ragwort introductions to North America and New Zealand originated. However, as outlined above, the primary focus of this study was on comparing populations from the introduced range that have experienced successful

biological control with populations that have not been exposed to biological control. A complete list of the populations included in the experiment is provided in Table 2 in Appendix.

In June 2005, seeds of the 32 populations were weighed and sown individually into 3 × 3 cm pots filled with peat soil. Since this took a relatively long time, seeds for the field block 1 were sown first, followed by the seeds for field block 2 and 3. Pots were put in the fridge at 7°C until all seeds were sown for an entire block in order to synchronize seedling development. Pots for block 1 were transferred to the greenhouse on 4 June, of block 2 on 7 June and of block 3 on 12 June. The average daily temperature in the greenhouse was 24°C with a maximum of 38°C on sunny days and 19°C during night. Incident light was supplemented by high-pressure sodium lamps (type SGR, Son-T-400 W [Philips, Zürich, Switzerland]). The pots were regularly watered. Due to poor germination rate, additional seeds from 24 populations were mass-germinated in Petri dishes on moist filter paper, this time without weighing the seeds before germination. The Petri dishes were placed beside the trays in the greenhouse. When the cotyledons appeared, the seedlings were transplanted into 3 × 3 cm pots filled with peat soil. Fifty-two of the 480 seedlings used in the field experiment (see below) originated from the mass-germination in Petri dishes. When the seedlings had reached the 2- to 4-leaf stage, they were transferred individually to larger pots (9 cm Ø) filled with a mix of peat soil and sand (ratio 1:1).

Field experiment

As we used populations from the introduced (New Zealand, North America) and native (Switzerland and The Netherlands) range, and in order to minimize the risk of their unwanted spread, the experimental garden was set up only in the native range. Moreover, the plants were harvested during the flowering stage in order to prevent seed set.

In July 2005, a meadow adjacent to CABI Europe-Switzerland Centre in Delémont, Switzerland, was mown to obtain a uniform vegetation height. The *J. vulgaris* plants were arranged in a randomized block design, with one plant of each of five families of the 32 populations planted in each of three blocks (resulting in a total of 480 plants). Plants assigned to the same block were transplanted within 48 h, but the different blocks were set up at different dates to allow all plants (also those originating from mass germination) to reach at least the six-leaf stage. Within each block, populations were arranged in a regular design by planting one plant of a randomly chosen population from the introduced range without *L. jacobaeae* biological control history, followed by one plant of a randomly chosen population from the introduced range with *L. jacobaeae* biological control history and one plant of a randomly chosen population from the native range. Plants were spaced 1 m apart, with 5 m distance between the blocks. In addition, a row of plants from a random mix of families was added along the periphery of each block to minimize edge effects. Dead plants were replaced within the first week after transplanting. Molluscicide granules (Blaukorn®, Pluess-Stauffer AG, Oftringen, Switzerland) were brought out around all plants to reduce mortality due to mollusc attack. The vegetation around the plants was left undisturbed. Since the summer of 2005 was very hot and dry, all plants were watered during the first week after transplanting.

One week after transplanting the plants into the field blocks, we measured the length of the longest leaf as well as the total number of green leaves.

Plants were first measured at the age of 8–10 weeks, and then re-measured at a monthly interval until the end of the growing season (Table 3 in Appendix). The length of the longest leaf and number of leaves was multiplied to obtain an estimate of rosette size that

has been shown to be significantly correlated with above-ground biomass in *J. vulgaris* (Wesselingh 1995). RGR was calculated either as the daily increase in biomass between size measurement 3 and size measurement 5 (same calendar dates for all blocks), or during a comparable period of time after transplanting the plants into the field, i.e. in block 1 from size measurement 1 to size measurement 3, in block 2 from size measurement 2 to size measurement 4, and in block 3 from size measurement 3 to size measurement 5 (Table 3 in Appendix). In both cases, RGR was calculated as RGR = (log (rosette size at the end of the period)—log (rosette size at the beginning of the period))/number of days. As the two measurements of RGR yielded similar outcomes, only the results of RGR based on time after transplanting are presented.

Both *J. vulgaris* and *L. jacobaeae* occur naturally at the field site. As the local population of *L. jacobaeae* did not provide sufficient individuals for the experiment, some 300 additional adults were collected on 13 September in a grassland some 50 km away from the experimental site at St-Imier, Canton Bern, and 100 adults each (sex ratio 1:1) released in the three blocks of the field experiment. Together with the naturally occurring population this resulted in an overall density of $\sim$1 adult *L. jacobaeae* per plant. Two other *Longitarsus* species feeding on *J. vulgaris* occur naturally in the area of the study site, but their densities during the experiment were much lower than those of *L. jacobaeae* ($\sim$1 adult per 10 plants) Feeding damage by adult *L. jacobaeae* was estimated by counting the total number of shot-holes on all green leaves at the end of the season after the first severe frost, i.e. when most adult *L. jacobaeae* had died (Table 3 in Appendix). Previous investigations with two native and two introduced populations indicated that number of shot-holes is significantly related to total area eaten and does not differ among populations (ANCOVA with population: $F_{3,32} = 0.67$, $P > 0.5$; number of shot-holes: $F_{1,32} = 32.0$, $P < 0.001$; population x number of shot-holes interaction: $F_{3,32} = 1.09$, $P > 0.3$), and that the total number of shot holes counted at the end of the season reflects the cumulative damage plants experience during the rosette stage reasonably well (Stastny et al. 2005).

In spring 2006, the plants from block 1 were harvested to assess the number of larvae mining inside the roots. The number of larvae was positively correlated with the number of shot-holes recorded in the previous autumn (Spearman's rank correlation $r = 0.221$, $P = 0.014$, $N = 123$). Because the data for number of larvae could not be normalized and were restricted to just one plot, we decided to only calculate ANOVAs using adult feeding damage as response variable. In summer 2006, the plants from block 2 and 3 were harvested at a standardized phenological stage, i.e. when the first flowers started losing their petals. The number of flowers (flowers with yellow or brown petals plus flower heads that had already lost their petals) were counted, the plants dried at 60°C during 72 h, and below- and above-ground biomass determined. Since the analyses of below-, above-ground and total biomass revealed comparable results, only the analysis of total biomass will be presented.

Leaf physiology and chemistry

For the analysis of LDMC, PAs and soluble phenolics, the 5th fully developed leaf from top was collected on 3–5 October 2005 from all plants of block 1. In twenty cases, an individual plant was missing and a replicate of the same family was sampled from block 2 or 3. Leaf samples were weighed immediately after clipping, then dried for 3 days at 40°C, reweighed and stored at −20°C until further analysis. LDMC was used as a proxy for leaf palatability, since it is closely related to the total amount of phenolics (which includes lignin and tannins; Elger and Willby 2003).

PAs were extracted by acid–base extraction (Hartmann and Zimmer 1986). PA composition of the plants was determined with GC-FID (Vrieling and de Boer 1999). Heliotrine was used as an internal standard to calculate individual PA concentrations, which were then combined to calculate total PA concentration. The concentrations of the individual PAs were then pooled to determine total PA concentration, since *L. jacobaeae*, similarly to *Tyria jacobaea* (Macel et al. 2002), does not appear to distinguish among individual PAs (Schaffner, unpublished results).

Analysis of soluble phenolics was carried out with those 120 plants from which enough plant material was left over after the analysis of PAs. For the extraction of soluble phenolics, 25 mg of dry leaf material was added to 5 ml 50% MeOH and slowly shaken for 66 h. After centrifugation (10 min at 2,000 rpm), 0.5 ml was transferred to a test tube and filled up with distilled water to 2.8 ml. Then 0.2 ml Folin–Ciocalteu's reagents (Merck) were added and mixed well. After 3 min, 1 ml 0.5 M NA_2CO_3 was added and again mixed well and after 8 min the absorption was measured at 725 nm. A calibration curve was made with a standard solution of chlorogenic acid (0.3 mg per ml in 50% MeOH). Phenolic concentration of the samples was calculated in "mg of chlorogenic acid equivalent" by correcting for the real amount of dry material used (Singleton et al. 1999).

Statistical analyses

To partition the variance of RGR, number of flowers, number of shot-holes and biomass at harvest between native and introduced populations, we included the following terms in hierarchical analyses of variance: block (random factor), origin (native versus introduced range; fixed factor), and population and family nested within population as random factors in the model. To assess differences among introduced populations, hierarchical analyses of variance were carried out including block (random factor), region within the introduced range (i.e. North America and New Zealand; fixed factor), biological control history (with or without *L. jacobaeae* biological control history; fixed factor), region x biological control history interaction, and population and family nested within population as random factors. For the analyses of number of shot-holes, rosette size in autumn (size measurement 5) was included in the models as a covariate. Since LDMC and concentrations of PAs and soluble phenolics were only assessed from one replicate per family, analyses of variance were calculated as described above, but without block and family as random factors. To meet the assumptions of analysis of variance, data on rosette size, number of flowers and concentrations of PAs and soluble phenolics were natural log transformed prior to analysis.

Because RGR was calculated differently among the three blocks (either at different calendar dates or at different periods after transplanting; see above), we also calculated ANOVA models that included the interactions between block and the fixed factors. However, all interactions between block and fixed factors turned out to be non-significant and were therefore excluded from the final model.

Mortality during the first growing season and during winter (using data from blocks 1–3) as well as probability of flowering in the second season (using data from blocks 2 and 3; Table 3 in Appendix) were assessed using analysis of deviance based on the models described above.

Correlations among RGR, PAs, soluble phenolics, LDMC and relative number of shot-holes (shot-holes divided by rosette size in autumn) were assessed using Pearson correlation analysis, with P values adjusted for multiple comparisons using sequential Bonferroni test. To assess the relationship between latitude of population origin and RGR, number of flowers or concentrations of PAs and soluble phenolics, we conducted

regression analyses using the population means for these parameters; the regressions were calculated for the whole set of introduced populations as well as separately for the populations from New Zealand and for those from North America. All statistical analyses were conducted using the SPSS statistical package, version 16.0.

Results

Differences between native and introduced populations

Populations in the introduced range had 1.5-fold higher concentrations of PAs (introduced populations: 2.37 ± 0.16 mg/g dry weight; native populations: 1.52 ± 0.35 mg/g dry weight; $F_{1,30} = 6.636$, $P = 0.015$) and produced twice as many flowers than populations from the native range (introduced populations: 493.61 ± 39.97; native populations: 225.43 ± 39.76; $F_{1,30} = 9.158$, $P = 0.005$). RGR, concentration of soluble phenolics, LDMC, number of shot-holes and biomass at harvest did not differ between native and introduced populations ($P > 0.1$).

A total of 51 out of the 480 plants died during the first season, and another 103 plants during the winter. Mortality did not differ between native and introduced populations neither during the first growing season nor during the winter ($P > 0.2$). Of the 203 surviving plants in blocks 2 and 3, 179 flowered in summer 2006, while 24 remained in the rosette stage. Flowering probability did not differ between native and introduced populations ($P > 0.5$).

Differences among introduced populations

The analyses of the concentrations of PAs and of soluble phenolics both revealed a significant region by biological control history interaction among introduced populations (Table 1). In New Zealand, populations with exposure to biological control had lower levels of generalist-deterrent pyrrolizidine alkaloids (PAs) and soluble phenolics in New Zealand than populations without exposure to biological control, while the opposite pattern was found among North America populations (Fig. 1). The relative number of shot-holes also differed significantly between populations with different *L. jacobaeae* biological control history. However, contrary to our predictions, introduced populations which were exposed to *L. jacobaeae* biological control were more attacked than populations which had never experienced biological control by *L. jacobaeae* (Table 1; Fig. 2). No differences were found between the two introduced regions and between populations with and without biological control history for RGR, LDMC, biomass at harvest and number of flowers (Table 1).

Latitude of population origins had no effect on population means for RGR, number of flowers and concentrations of PAs and soluble phenolics when populations from New Zealand were analysed alone or in combination with the North America populations. In contrast, latitude exhibited a significant negative effect on concentrations of PAs (Fig. 3; $\ln(y) = -0.041x + 2.95$, $r^2 = 0.36$, $P = 0.017$) and of soluble phenolics of North American populations ($\ln(y) = -0.021x + 3.46$, $r^2 = 0.29$, $P = 0.037$).

Mortality during the first growing season did not differ between North American and New Zealand populations, nor between populations with and without biological control history ($P > 0.1$). Winter mortality was higher in populations from North America than in populations from New Zealand ($F_{1,27} = 6.17$, $P < 0.05$), but did not differ among

Table 1 ANOVA and ANCOVA of the effects of region and biological control history on plant and herbivore response variables

Source of variation	Region			Biocontrol history			Region by biocontrol history			Rosette size inautumn (Covariate)		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Plant vigour												
Relative growth rate	1,21	0.728	0.403	1,21	0.085	0.774	1,21	0.531	0.474		NI	
Biomass at harvest	1,21	0.063	0.804	1,21	0.675	0.422	1,21	0.523	0.478		NI	
Number of flowers	1,21	0.322	0.577	1,21	0.489	0.493	1,21	0.080	0.781		NI	
Leaf physiology												
Leaf dry matter content	1,22	0.010	0.980	1,22	0.470	0.502	1,22	0.160	0.695		NI	
Plant defence traits												
Pyrrolizidine alkaloids	1,22	0.148	0.704	1,22	0.160	0.693	1,22	7.642	0.011		NI	
Soluble phenolics	1,14	1.301	0.272	1,14	0.023	0.883	1,14	9.708	0.008		NI	
Insect damage												
Number of shot-holes	1,20	1.125	0.093	1,20	13.622	0.002	1,20	0.734	0.402	1,276	105.34	0.001

All analyses are based on introduced *J. vulgaris* populations only. *NI* covariate not included in the model

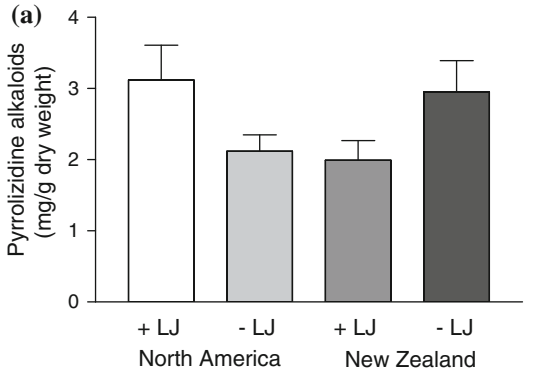
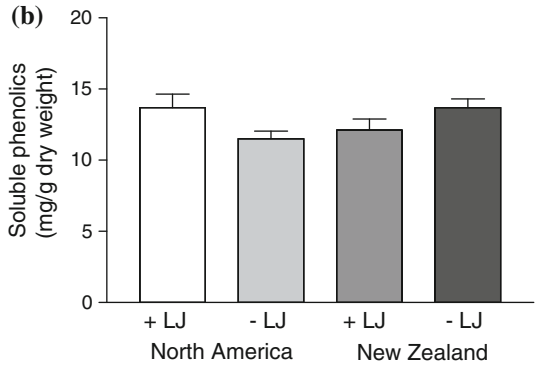


Fig. 1 Concentrations of **a** pyrrolizidine alkaloids and **b** soluble phenolics (mean + SE) in introduced populations from North America and New Zealand. +LJ, populations with exposure to the biological control agent *L. jacobaeae*; −LJ, populations without exposure to *L. jacobaeae*



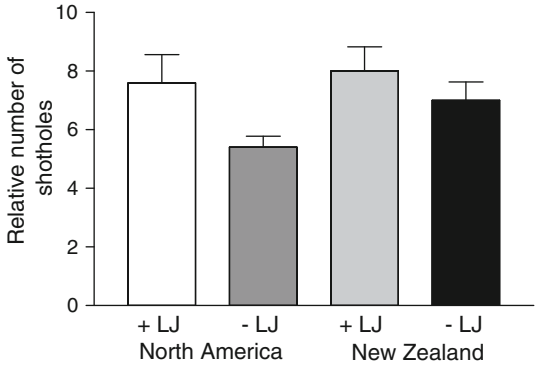


Fig. 2 Number of shot-holes corrected for rosette size in autumn (mean + SE) in plants of introduced populations from North America and New Zealand. +LJ, populations with exposure to the biological control agent *L. jacobaeae*; −LJ, populations without exposure to *L. jacobaeae*

populations with and without biological control history ($P > 0.8$). Flowering probability differed neither between North American and New Zealand populations nor between populations with and without biological control history ($P > 0.2$).

Correlations among plant traits

The concentration of soluble phenolics was positively correlated with the concentration of PAs ($r = 0.182$; $P = 0.030$; $N = 124$) and with LDMC ($r = 0.229$; $P = 0.013$; $N = 117$), but not with RGR or relative number of shot-holes (both $P > 0.1$). No

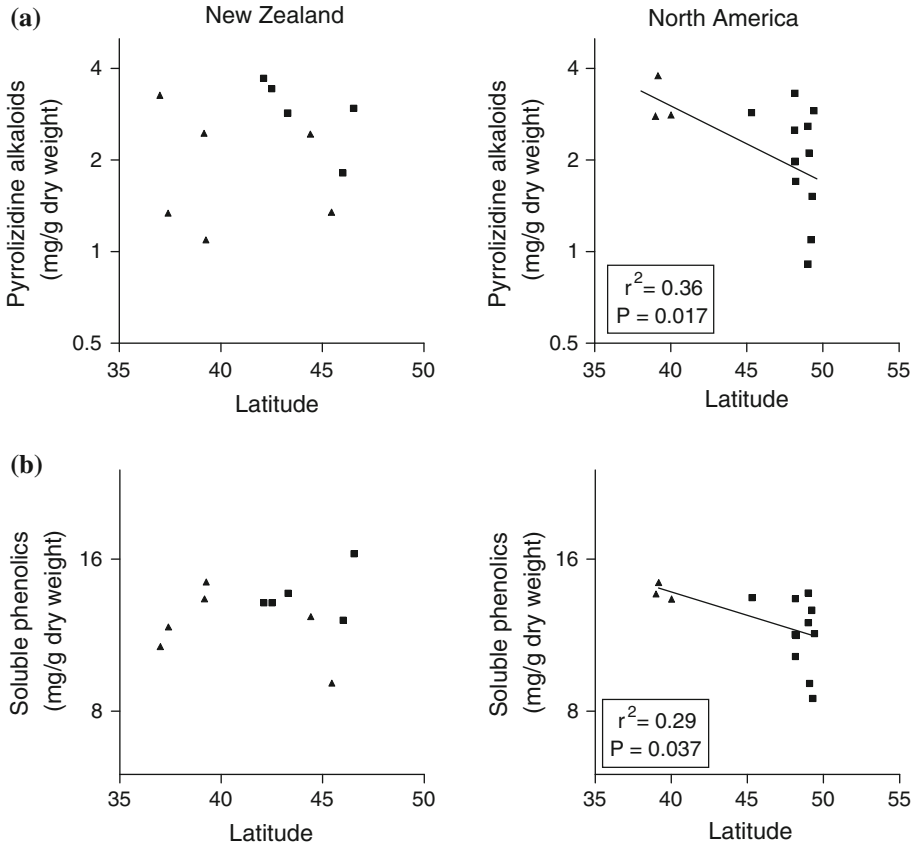


Fig. 3 Regression of concentrations of **a** pyrrolizidine alkaloids and **b** soluble phenolics on latitude among New Zealand (*left side*) and North American (*right side*) populations. *Squares* indicate populations without exposure to the biological control agent *L. jacobaeae*, *triangles* populations with exposure to *L. jacobaeae*. *Lines through symbols* indicate significant effect of latitude

significant correlation was found among RGR, concentrations of PAs, LDMC and relative number of shot-holes (all $P > 0.1$). However, plants with higher PA concentrations tended to receive more feeding damage in absolute terms ($r = 0.145$; $P = 0.088$; $N = 140$).

Discussion

In the native range, enemy pressure and selection on resistance traits have been found to vary greatly, even among closely located populations (Maron and Vilà 2008 and references therein). Moreover, exotic plant species are introduced into a heterogeneous environment harbouring diverse recipient communities, and different genotypes may arrive in different regions or habitats, which may ultimately influence the direction and speed of evolutionary change in introduced populations. Thus, it can hardly be expected that all populations universally evolve from well defended to poorly defended where they are introduced.

We studied variation in life-history traits among introduced populations of *J. vulgaris* from two different parts of the introduced range and compared populations with and

without exposure to *L. jacobaeae*, the biological control agent that is considered to be at least partly responsible for the regional decrease of this invasive weed. So far, this approach has rarely been used to explore evolutionary change in introduced species (but see Zangerl and Berenbaum 2005; Maron and Vilà 2008; Handley et al. 2008; Müller-Schärer and Schaffner 2008), although it can reduce problems encountered when comparing populations from the native and the introduced range (Colautti et al. 2004).

Based on our results, one might assume that (re-)exposure of *J. vulgaris* to *L. jacobaeae* has led to opposite evolutionary trajectories in resistance traits in New Zealand and North America. In New Zealand, concentrations of PAs and soluble phenolics were lower in populations that have been exposed to *L. jacobaeae*. A reduced concentration of PAs is consistent with our hypothesis that (re-)exposure to specialist natural enemies should reduce qualitative defence traits if they can be used by specialists for locating host-plants or increasing their own survival (Müller-Schärer et al. 2004). *Longitarsus jacobaeae* sequesters PAs (Dobler et al. 2000) and may therefore benefit from feeding on PA-rich plants. Field studies indicate that plants with higher PA concentration tend to receive more *L. jacobaeae* damage in absolute terms (Stastny et al. 2005; this study). However, *L. jacobaeae* also responds positively to plant size (Windig 1993). When *L. jacobaeae* feeding damage is corrected for plant size, the relationship between feeding damage and PA concentration becomes non-significant (this study) or even negative (Vrieling and van Wijk 1994; Stastny et al. 2005). Therefore, the direct role of PAs in host-selection by *L. jacobaeae* remains to be shown.

While alkaloids have been put forward as defence chemicals acting primarily against generalist natural enemies, soluble phenolics have been shown to affect preference and performance of generalist as well as specialist herbivores (Schoonhoven et al. 1998). It is unknown to what extent chlorogenic acid or any other soluble phenolic present in *J. vulgaris* affects preference or performance of *L. jacobaeae*. Yet, the positive correlation between concentrations of soluble phenolics and PAs found in this study suggests that, even in the absence of a direct effect of soluble phenolics on preference or performance of *L. jacobaeae*, selection by the biological control agent on PA concentration may also affect concentration of soluble phenolics.

In North American populations, the pattern in resistance traits appeared to be opposite of what was found in New Zealand populations. It should be noted, however, that the sampling of the populations in North America was constrained by the fact that successful biological control by *L. jacobaeae* has been largely restricted to southwestern and western parts of the USA (Julien and Griffiths 1998), while populations without biological control history are located in the northern part of the USA and in Canada (Table 2 in Appendix). Because concentrations of PAs and soluble phenolics in North American populations were found to be significantly related with latitude of population origin, our experimental design did not allow testing of whether the pattern found in North America is indeed due to different biological control history, or whether it is due to confounding geographical variation. Clinal variation in life-history traits has been reported for a range of invasive species (Weber and Schmid 1998; Kollmann and Banuelos 2004; Maron et al. 2004). A disjunct distribution of populations that have been exposed to biological control and populations that have remained free from specialist herbivory is known for various other weed systems, including *Hypericum perforatum* L. (Vilà et al. 2003). In New Zealand, however, the *J. vulgaris* populations with and without biological control history were collected within approximately the same latitudinal range (Table 2 in Appendix).

Based on the specialist-generalist dilemma, we predicted that populations in the introduced range that were re-exposed to *L. jacobaeae* would evolve increased levels of

resistance against this biological control agent. However, LDMC was not increased, despite the fact that this trait is considered to incur some level of protection against specialist herbivores (Elger and Willby 2003). Moreover, populations with biological control history had more feeding damage by *L. jacobaeae*, when corrected for rosette size, than populations without biological control history, suggesting that *J. vulgaris* populations that were re-exposed to *L. jacobaeae* have evolved reduced resistance to this herbivore species. An alternative explanation of this counterintuitive result is that *L. jacobaeae* was released but failed to establish in some of the *J. vulgaris* populations that were considered to be free of biological control history. If so, then these *J. vulgaris* populations might have already had higher levels of resistance at the time of re-exposure to *L. jacobaeae* than those populations in which *L. jacobaeae* succeeded in building up outbreak population densities. Also, it is unknown to what extent other biocontrol agents released in New Zealand and North America or generalist arthropods that have been recorded feeding on *J. vulgaris* in the introduced range (Frick 1972) impose some level of selection on traits of invasive *J. vulgaris* populations.

We found considerably higher PA concentrations and higher reproductive output in *J. vulgaris* populations from the introduced range than in populations from the native range, which is in line with the results of previous studies (Joshi and Vrieling 2005; Stastny et al. 2005). Three of the six populations from the native range were of the erucifoline chemotype, while the other three populations from the native range and all populations from the introduced range were of the jacobine chemotype (C. Rapo, unpublished results). The three populations of the erucifoline chemotype had lower PA concentrations than the three populations of the jacobine chemotype from the native range; however, when comparing the three native populations of the jacobine chemotype with those of the introduced range, no significant difference in PA concentration was found. These findings are in line with the results of the study by Joshi and Vrieling (2005) who found generally higher PA concentrations in the jacobine chemotype than in the erucifoline chemotype, but similar PA concentrations in native and in introduced populations of the jacobine chemotype. Joshi and Vrieling (2005) suggested that the erucifoline chemotype has either not been introduced to most of the invasive areas or has been selected against in the new environments. Assessing the genetic relationship between native and introduced populations of *J. vulgaris* may clarify whether *J. vulgaris* has indeed undergone rapid evolution after its introduction into New Zealand and North America.

Opposite to previous studies (Joshi and Vrieling 2005; Stastny et al. 2005), RGR, rosette size in autumn 2005 and biomass at harvest did not differ between native and introduced populations. One explanation for the lack of differences in size may be that in our study the rosettes were transplanted into dense vegetation and were therefore exposed to high interspecific competition, while Joshi and Vrieling (2005) grew the plants in absence of competition and Stastny et al. (2005) in a disturbed field with low competition. Differences in growth rate between plants from invasive populations and from native populations can be context-dependent; Leger and Rice (2003) found that plants from invasive populations of *Eschscholzia californica* Cham grew larger than those from native populations when grown in a competition-free environment, but no differences were found when plants were grown with competition from other plant species. Alternatively, the non-significant difference in plant size between populations from the native and the introduced range in our experiment, which was carried out in a field where *J. vulgaris* occurs naturally, may also be due to negative feedbacks of the native soil which may have accumulated *Jacobaea* pathogens (Bezemer et al. 2006).

Concluding remarks

We confirmed clear differences in PA concentration and reproductive output between native and introduced populations of *J. vulgaris*, which is in line with expectations based on the refined EICA hypothesis (Müller-Schärer et al. 2004; Joshi and Vrieling 2005). However, our study provides little evidence for a rapid evolutionary adaptation of *J. vulgaris* populations in the introduced range to the biological control agent *L. jacobaeae*, despite the significant impact of this specialist herbivore on the population dynamics of the target weed in the introduced range (McEvoy et al. 1991). The small differences between introduced populations with and without exposure to *L. jacobaeae* and the larger differences between native and introduced populations may result from different time scales available for selection to act, with *J. vulgaris* introduced in different parts of the world some 100–130 years (~50 generations) and *L. jacobaeae* only some 20–40 years ago (10–15 generations). Theoretically, rapid evolution in invasive *J. vulgaris* populations re-exposed to *L. jacobaeae* seems possible even after a relatively short period of time, since exotic species exposed to new environments have been repeatedly shown to undergo rapid evolution (Stockwell et al. 2003; Zangerl and Berenbaum 2005), and since outbreak densities of *L. jacobaeae* are likely to have exerted or still exert strong directional selection pressure on exotic *J. vulgaris* populations. However, a rapid evolutionary response by *J. vulgaris* in the presence of *L. jacobaeae* may be constrained by pleiotropic effects (Mitchell-Olds 1996), since the concentrations of PAs and soluble phenolics are correlated with other plant traits, such as leaf morphology (Rapo, unpublished results) and shoot:root ratio during the seedling stage (Schaffner et al. 2003). We propose that a detailed knowledge of the variation among introduced populations in terms of their biological control history constitutes an excellent but yet underappreciated framework to study the evolutionary ecology of invasive plants.

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Appendix

See Tables 2 and 3.

Table 2 Origin of the populations of *Jacobaea vulgaris* included in the common garden experiment, and presence/absence of the biological control agent *Longitarsus jacobaeae* (in Chereng probably the sibling species *L. flavicornis*)

Pop.	Country	Site of seed collection	Longitude	Latitude	*L. jacobaeae*
Invasive populations without exposure to *L. jacobaeae*					
1	NZ	Maruia	E172,13	S42,11	–
2	NZ	Craigieburn	E171,40	S42,50	–
3	NZ	Cook River Flat	E170,00	S43,30	–
5	NZ	Halfmoon Bay	E168,08	S46,54	–
6	NZ	Southland 2	E169,00	S46,00	–

Table 2 continued

Pop.	Country	Site of seed collection	Longitude	Latitude	*L. jacobaeae*
7	Canada	Vancouver Island (BC)	W125,3	N48,9[a]	–
8	Canada	Laidlaw (BC)	W121,26	N49,23	–
9	Canada	Peters Road (BC)	W121,0	N49,0[a]	–
10	Canada	Chute Lake (BC)	W119,35	N49,30	–
11	Canada	Forestry Road (BC)	W119,0	N49,2[a]	–
12	Canada	Big Meadow (BC)	W119,0	N49,1[a]	–
13	USA	C-spur (Montana)	W114,53	N48,18	–
14	USA	Island Lake (Montana)	W109,26	N45,31	–
15	USA	Site 2 (Montana)	W114,47	N48,20	–
25	USA	No Bear	W114,53	N48,14	Released when seeds were collected
26	USA	Surprise Hill (Montana)	W114,56	N48,15	Released when seeds were collected
27	USA	Little Wolf 03	W114,53	N48,17	Released when seeds were collected
Invasive populations with exposure to *L. jacobaeae*					
16	NZ	Mangatoki	E174,04	S39,18	+
17	NZ	Opunake	E173,51	S39,27	+
18	NZ	Tauranga Bay	E176,10	S37,42	+
20	NZ	Landsborough	E169.09	S44,42	+
23	NZ	Whapitu	E174,30	S37,00	+
24	NZ	Southland 1	E169,00	S45,45	+ (+ *Tyria jacobaea*)
28	USA	Mendocino (California)	W123,47	N39,18	+
29	USA	Del Norte (California)	W123,0	N39,5[a]	+
30	USA	Humboldt (California)	W118,31	N40,02	+
Native populations with *L. jacobaeae*					
31	CH	Mettembert (Jura)	E07,20	N47,22	+
32	CH	St-Imier (Bern)	E07,00	N47,09	+
33	CH	L'Himelette (Bern)	E07,03	N47,08	+
34	NL	Meijendel	E04,20	N52,09	+
35	NL	Leiden	E04,30	N52,09	+ (+ *Tyria jacobaea*)
36	F	Chereng	E03,21	N50,61	+ (*L. flavicornis?!*)

NZ New Zealand, *USA* United States of America, *CH* Switzerland, *NL* Netherlands, *F* France

[a] Approximate values for coordinates

Table 3 Timetable of the experimental tasks carried out in each of the three experimental blocks

Task	Block 1	Block 2	Block 3
Sowing date	5/6/05	7/6/05	12/6/05
Transplanting to garden	27–28/7/05	9–10/8/05	22–23/8/05
Number of transplanted seedlings	160	160	160
Size measurement 1	1–3/8/05	–	–
Size measurement 2	–	17/8/05	–

Table 3 continued

Task	Block 1	Block 2	Block 3
Size measurement 3	30/8–1/9/05	30/8–1/9/05	30/8–1/9/05
Size measurement 4	20–22/9/05	20–22/9/05	20–22/9/05
Sampling for chemical analyses	3–5/10/05	–	–
Size measurement 5	24–26/10/05	24–26/10/05	24–26/10/05
Number of shot-holes	27–28/10/05	9–10/11/05	15–25/10/05
Biomass at harvest	–	Summer '06	Summer '06
Number of flowers	–	Summer '06	Summer '06

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